potassium have also been shown in patients with MSK, potentially contributing to the pathogenesis of nephrolithiasis.[3]

This renal malformation is frequently associated with nephrocalcinosis and renal stones. In particular, MSK is associated with a 60% lifetime risk of renal stones, and the prevalence of MSK in patients with renal stones is significantly higher (8.5%, $P<0.01$) than in the control population (1.5%).[4]

As MSK was diagnosed only after the introduction of intravenous urography in the 1930s (Figure 1), and conventional computed tomography, which has been preferred since the mid-1990s, is not satisfactory for unmasking MSK, except when using multidetector-row computed tomography of high-resolution three-dimensional displays and late urographic images, 'there is a concrete possibility of this renal condition being forgotten in the future.'[2] Therefore, it seems to be even more mandatory not to miss MSK in reviews of renal stones.

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Piero Stratta[1], Roberta Fenoglio[1], Marco Quaglia[1], Elisa Lazzarich[1] and Andrea Airoldi[1]
[1]*Division of Nephrology and Transplantation, Department of Clinical and Experimental Medicine and International Research Centre Autoimmune Disease (IRCAD), Amedeo Avogadro University, Novara, Ospedale Maggiore della Carità, Novara, Italy*
Correspondence: *Piero Stratta, Division of Nephrology and Transplantation, Department of Clinical and Experimental Medicine and International Research Centre Autoimmune Disease (IRCAD), Amedeo Avogadro University, Ospedale Maggiore della Carità, Corso Mazzini 18, Novara 28100, Italy.*
E-mail: strattanefro@hotmail.com

Response to 'The missing medullary sponge kidney'

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I agree with Stratta *et al.*[1] that the association of various malformative disorders with medullary sponge kidney (MSK) is interesting, yet challenging. These various associations have led to the hypothesis that embryological nephronal maldevelopment may participate in the pathogenesis of this disease.[2] However, as stated in a very recent article published in this journal, the pathogenesis of MSK has not yet been fully elucidated.[2] Although multiple candidate genes have been proposed to play a role in the process of embryological development, none have been revealed to be directly involved in uteric-bud/metanephric-blastema interface disruption.[3–5] This notion is supported by a study on the kidneys of pre-term infants showing an abundance of calcification-promoting molecules, specifically osteopontin and hyaluronan, expressed at the luminal side of the renal tubular differentiating cells.[6]

There was no mention made of the role of MSK in my recent paper[7] because the true advancement in this field needs to be substantiated. This could be achieved by direct tissue examination of the renal papillary structure in affected patients for both the presence of undifferentiated embryonal tissue and for the expression of various potential genes participating in nephronal development. The metabolic abnormalities that were mentioned by Stratta *et al.*[1] are only associations that may contribute to kidney stone formation but are not causal in the pathogenesis of this disease.[8]

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Khashayar Sakhaee[1]
[1]*Department of Internal Medicine, Charles & Jane Pak Center for Mineral Metabolism & Clinical Research, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, USA*
Correspondence: *Khashayar Sakhaee, Department of Internal Medicine, Charles & Jane Pak Center for Mineral Metabolism & Clinical Research, University of Texas Southwestern Medical Center at Dallas, 3523 Harry Hines Blvd., Dallas, Texas 75390-8885, USA.*
E-mail: khashayar.sakhaee@utsouthwestern.edu

It takes two to Twist

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To the Editor: It is with interest that we read the report of Sun *et al.*[1] on how hypoxia-inducible factor-1α (HIF-1α) induces *TWIST*1 expression in human tubule cell lines, providing a model for hypoxia-induced renal fibrosis. Their findings complement two earlier papers linking hypoxic signaling to *TWIST*1 expression. In a functional screen in *Caenorhabditis elegans*, TWIST1 was identified as a HIF target, and in human cancer cells, hypoxia induces *TWIST*1 expression and epithelial–mesenchymal transition.[2–4] Importantly, silencing of *TWIST*1 attenuates metastatic cancer outgrowth in xenograft models.[4] Taken together, these

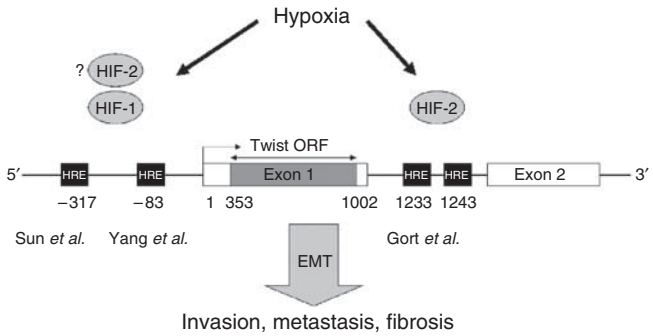

Figure 1 | Schematic representation of the *TWIST1* genomic structure and the putative hypoxia-responsive elements (HREs) involved in *TWIST1* regulation by hypoxia-inducible factor (HIF) proteins. Exons (white boxes), the coding sequence (gray box), and the HREs (black boxes) are shown here. Nucleotide positions of functional HREs in the TWIST1 genomic locus bound by HIF-1α or HIF-2α proteins in different systems are indicated (see text for details). EMT, epithelial–mesenchymal transition.

earlier studies had already established an important role for hypoxia-induced TWIST1 expression.

Whereas both Yang *et al*. and Sun *et al*. observe a role for HIF-1α in regulating *TWIST*1 through hypoxia-responsive elements in the *TWIST*1 proximal promoter, we showed a regulation of *TWIST*1 by HIF-2, the HIF-1 ortholog. Importantly, HIF-2α induces *TWIST*1 expression using a distinct intronic hypoxia-responsive element rather than through the proximal *TWIST*1 promoter.[3] Although we excluded an involvement of HIF-1 in both intronic and proximal hypoxia-responsive element regulation of *TWIST*1, other studies have not addressed a role for HIF-2.

Therefore, it seems that hypoxic *TWIST*1 induction is regulated by both HIF-1α and HIF-2α proteins through distinct regulatory elements in a tissue-specific manner (Figure 1). It is clear that the hypoxic regulation of *TWIST*1 plays a key role in hypoxic epithelial–mesenchymal transition induction and metastases formation. It will be interesting to learn the mechanistic and clinical importance of the HIF-1- versus the HIF-2-mediated regulation of *TWIST*1 in disease processes.

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Arjan J. Groot[1], Paul J. van Diest[1] and Marc A. Vooijs[1]

[1]*Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands*

Correspondence: *Marc A. Vooijs, Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands. E-mail: m.vooijs@umcutrecht.nl*

Response to 'It takes two to Twist'

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We thank Vooijs *et al.* for their interest and comments about our paper.[1] Vooijs *et al.* had found that hypoxia induces Twist1 expression in a hypoxia-inducible factor (HIF)-2α-dependent manner and that intronic hypoxia response elements of Twist1 are regulated by HIF-2α.[2] In our study, direct transcriptional activation of Twist by HIF-1α was found to promote epithelial-to-mesenchymal transition (EMT) in tubular cells.[3] Yang *et al.*[4] also showed that HIF-1α directly regulates the expression of Twist by binding to hypoxia-responsive elements in the Twist proximal promoter, resulting in EMT and metastatic phenotypes. Thus, *Twist* may be the target gene of both HIF-1α and HIF-2α. However, we think the role of Twist in hypoxia-induced renal EMT and fibrosis is mediated only by HIF-1α, not HIF-2α.

The expressions of both HIF-1α and HIF-2α are tissue- and cell type–specific. HIF-1α is ubiquitously expressed, whereas HIF-2α expression is more restricted. HIF-2α has been found in hepatocytes, cardiomyocytes, glial cells, type II pneumocytes, and endothelial cells. In addition, it has been reported that HIF-1α and-2α are also expressed in different renal cell populations.[5] HIF-1α is mainly induced in tubular cells as well as in proximal tubules, distal tubules, and connecting tubes. Although HIF-2α is not expressed in tubular cells, it is expressed in the endothelial cells of a small subset of glomeruli and in peritubular endothelial cells and fibroblasts. The selective induction of HIF-1α and -2α in different types of cells in the kidney should use different aspects of hypoxic response pathways in a cell type–specific or temporospatial manner. It would be interesting to further investigate the potential roles of the HIF-2α in hypoxic kidney.

In conclusion, although hypoxic Twist induction is regulated by both HIF-1α and HIF-2α in some specific cell types, including cancer cells, we feel that Twist-induced renal EMT and fibrosis under hypoxia are dominantly mediated by an HIF-1α-dependent pathway.

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Shiren Sun[1,2], Xiaoxuan Ning[3], Yongzhan Nie[2], Yuanyuan Lu[2] and Daiming Fan[2]

[1]*Department of Nephrology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi, China;* [2]*State Key Laboratory of Cancer Biology, Department of Nephrology, Xijing Hospital, Fourth Military Medical*